

BLOOD COMPOSITION AND MILK SECRETION

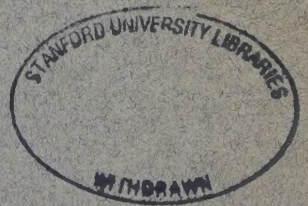
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Blood Glucose and Lactic Acid in Relation to Milk Secretion.†

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The recent *in vitro* synthesis of lactose by Petersen and Shaw¹ from lactic acid and glucose and the demonstration by Graham² of the loss of lactic acid from the blood traversing the mammary gland indicates that lactose is synthesized in the gland from lactic acid and glucose of the blood.

Kaufman and Magne³ found that in lactating animals the mammary venous blood contained less glucose than the jugular blood. Loss of glucose from the blood in passing through the lactating mammary gland has also been shown by Blackwood and Stirling,⁴ Lintzel,⁵ and Graham, Jones and Kay.⁶ The latter workers reported that the amount of glucose taken out of the blood was proportional to the level of glucose in the arterial blood and to the milk yield per day.

Several workers have demonstrated reduced blood and milk sugar

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* The data in this paper are from a thesis presented by J. C. Shaw in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, Minnesota Agricultural Experiment Station.

¹ Petersen, W. E., and Shaw, J. C., *Science*, 1937, **86**, 398.

² Graham, W. R., Jr., *J. Biol. Chem.*, 1937, **122**, 1.

³ Kaufman, M., and Magne, H., *Compt. Rend. Acad. Sci. Paris*, 1906, **143**, 779.

⁴ Blackwood, J. H., and Stirling, J. D., *Biochem. J.*, 1932, **26**, 357.

⁵ Lintzel, W., *Z. Zücht., B.*, 1934, **29**, 219.

⁶ Graham, W. R., Jr., Jones, T. S. G., and Kay, H. D., *Proc. Roy. Soc. London*, Series B, 1936, **120**, 330.

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by injection of insulin into cows, goats and sheep. Brown, Petersen and Gortner⁷ reported that reducing the blood sugar to 17 mg % resulted in a drop in milk sugar to about one-fourth normal.

It has been much more difficult, however, to increase the lactose by increasing the blood sugar. Whitnah, Riddell and Hodgson⁸ obtained only a slight increase in the lactose of milk when the blood sugar was raised to a very high level by pumping glucose solution into the stomachs of lactating cows. Likewise Brown, Petersen and Gortner⁷ obtained only a very slight increase in lactose secretion by producing a hyperglycemic condition with intramammary duct injections of isotonic glucose. The sugar content of blood was raised to 300 mg % by Petersen and Boyd⁹ by infusion of isotonic solutions of glucose into the external pudic artery without any increase in the lactose of the milk.

In a further study of the relation of blood glucose to milk secretion, a study was made of the relation of the blood glucose absorbed by the mammary gland to the following: time following completion of last milking, arterial blood sugar level, and level of milk production. A further study was made of the use of lactic acid by the mammary gland and the total blood carbohydrate needed for the elaboration of milk sugar.

Arterial and venous blood samples were taken simultaneously from the internal iliac artery or the external pudic artery and the subcutaneous abdominal mammary veins respectively. The method proposed by Graham, Kay and McIntosh¹⁰ was followed in obtaining the arterial blood from the internal iliac. The method of Boyd and Petersen¹¹ was used in obtaining arterial samples from the same animal on several consecutive days.

Lactic acid was determined by West's¹² modification of the von Fürth-Charnass technic.

The method used for blood sugar was essentially that recommended by Shaffer and Somogyi.¹³ The blood filtrates were pre-

⁷ Brown, W. R., Petersen, W. E., and Gortner, R. A., *J. Dairy Sci.*, 1936, **19**, 147.

⁸ Whitnah, C. H., Riddell, W. H., and Hodgson, R. E., *J. Dairy Sci.*, 1933, **16**, 347.

⁹ Petersen, W. E., and Boyd, W. L., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 537.

¹⁰ Graham, W. R., Jr., Kay, H. D., and McIntosh, R. A., *Proc. Roy. Soc. London, Series B*, 1936, **120**, 319.

¹¹ Boyd, W. L., and Petersen, W. E., unpublished data, 1938.

¹² West, E. S., *J. Biol. Chem.*, 1931, **92**, 483.

¹³ Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

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TABLE I.
Glucose in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods.

Cow No.	Glucose level		Arterial Venous Difference, mg %	Remarks
	Arterial, mg %	Venous, mg %		
435	68.0	59.0	— 9.0	2 hr after milking
432	63.6	53.4	—10.2	
446	63.6	58.4	— 5.2	
577	76.0	71.0	— 5.0	
434	68.8	66.4	— 2.4	
			avg —6.36	
435	65.0	56.0	— 9.0	3 hr after milking
241	75.0	62.0	—13.0	
241	62.0	49.0	—13.0	
241	59.4	48.4	—11.0	
435	70.0	63.0	— 7.0	
435	64.0	59.0	— 5.0	
241	81.6	68.4	—13.2	
447	64.0	53.0	—11.0	
419	74.0	71.0	— 3.0	
433	56.0	50.0	— 6.0	
			avg —9.12	
241	73.0	55.0	—18.0	4 hr after milking
241	71.0	60.6	—10.4	
241	74.4	63.0	—11.4	
241	59.6	51.0	— 8.6	
578	52.0	45.6	— 6.4	
26	47.8	39.4	— 8.4	
423	51.2	47.8	— 3.4	
423	78.8	63.0	—15.8	
			avg —10.30	
435	58.0	44.0	—14.0	5 hr after milking
435	65.0	59.4	— 5.6	
241	73.2	64.0	— 9.2	
E-190	70.0	64.0	— 6.0	
435	60.0	56.0	— 4.0	
435	76.4	60.4	—16.0	
443	64.0	52.6	—11.4	
212	59.6	45.8	—13.8	
213	59.6	50.6	— 9.0	
			avg —9.87	
435	64.0	58.4	— 6.0	9 hr after milking
E-190	78.0	71.0	— 7.0	
235	76.0	65.6	—10.4	
			avg —7.8	
435	61.0	56.0	— 5.0	10 hr after milking
241	48.0	33.4	—14.6	
25	74.4	65.4	— 9.0	
A-7	72.0	61.6	—10.4	
586	78.0	63.0	—15.0	
			avg —10.80	
Avg	66.4	57.1	— 9.3	

pared by the Somogyi¹⁴ method. Sodium citrate was used as an anticoagulant. Considerable precaution was exercised in obtaining

¹⁴ Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

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the blood samples. In the event that there was apparent excitation of the animal the samples were discarded.

The data for 40 arterio-venous glucose differences are presented in Table I. With the possible exception of the first 2 hours following milking there was no apparent relation between the arterio-venous glucose difference and the period of time following the completion of the last milking. The average arterio-venous blood sugar loss was 9.3 mg %.

A study was made of the relation of the arterio-venous glucose difference to the arterial blood sugar level and to the level of milk production. The results are given in Figs. 1 and 2. In both comparisons the A-V differences are so widely scattered as to hardly justify the plotting of a curve. The same situation prevails in the data presented in Table II in which several experiments were conducted on the same animals over a period of a few weeks. With the same individuals, neither the arterial blood sugar level nor the milk yield was related to the arterio-venous glucose difference.

The glucose and lactic acid concentration of arterial and mammary venous bloods are presented in Table III. There was a loss of lactic acid in 15 of the 17 experiments confirming the work by Graham² on lactating goats. The amount of glucose absorbed by the lactating mammary was usually greater than that of the lactic acid. It will be noted that there is no apparent relationship between the glucose and lactic acid loss at any one time. The same thing will be observed in Graham's data. It is believed that this indicates a storage in the mammary gland of the precursors of lactose. Dur-

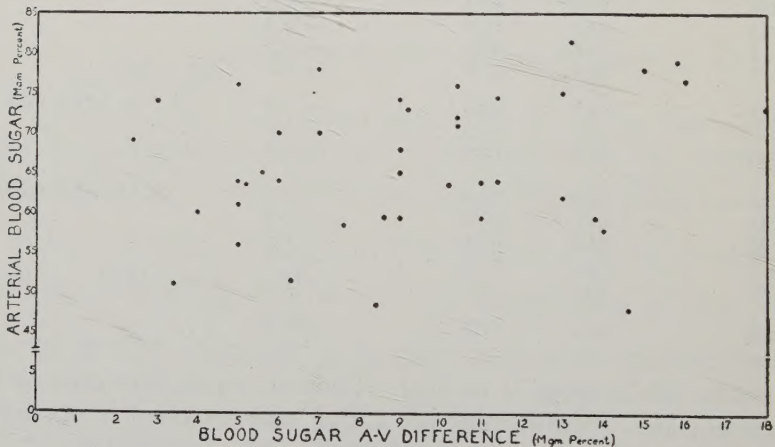


FIG. 1.
Relation of A-V difference to arterial blood sugar level.

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TABLE II.
Arterio-Venous Blood Sugar Difference in Relation to Arterial Level of Blood Sugar and to Level of Milk Production.

Cow No. 241		Cow No. 435	
Arterial Blood Sugar Level mg %	A-V Blood Sugar Difference mg %	Milk Yield lb	A-V Blood Sugar Difference mg %
75.0	-13.0	19.6	-5.0
74.4	-11.4	16.6	-5.6
73.2	-9.2	15.6	-9.0
73.0	-18.0	15.1	-9.0
71.0	-10.4	10.1	-5.0
62.0	-13.0	9.6	-4.0
59.6	-8.6	8.8	-7.0
59.4	-11.0	8.6	-7.6
48.0	-14.6		

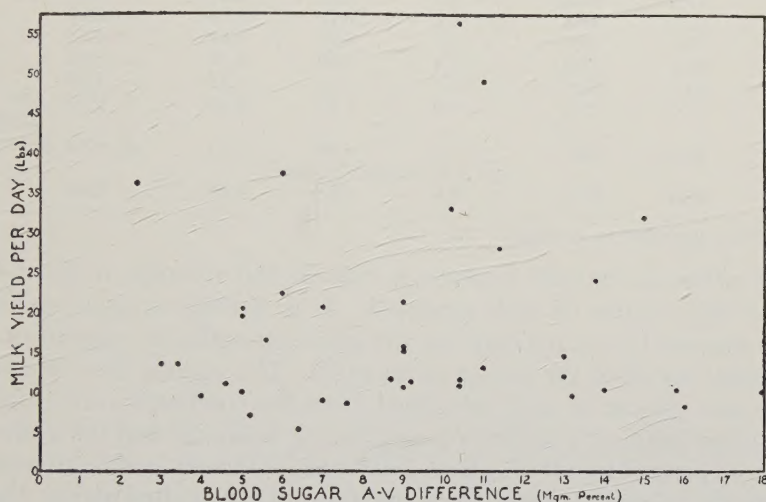


FIG. 2.
Relation of A-V difference to milk yield.

ing extreme excitement there is little or no loss in glucose and lactic acid and at times even a higher concentration in the venous blood of both substances.

The volume of blood flow as measured by the ratio of the calcium and fat removed by the gland to the percent of calcium and fat in the milk has been shown by Shaw and Petersen¹⁵ to be in the range of 391:1 and 344:1 respectively. The average arterio-venous glucose loss of 9.3 mg % as given in Table I when compared to the aver-

¹⁵ Shaw, J. C., and Petersen, W. E., *Proc. Am. Physiol. Soc.*, 50th Annual Meeting, Baltimore, Maryland, 1938, p. 183.

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TABLE III.
Glucose and Lactic Acid in Arterial and Mammary Venous Blood of Cows.

Glucose			Lactic acid		
Arterial mg %	Venous mg %	Difference mg %	Arterial mg %	Venous mg %	Difference mg %
63.6	53.4	—10.2	5.72	3.65	— 2.07
64.0	53.0	—11.0	7.13	8.68	+ 1.55
74.0	71.0	— 3.0	5.94	3.47	— 2.47
76.0	71.0	— 5.0	12.97	6.21	— 6.76
68.8	66.4	— 2.4	7.67	6.58	— 1.09
56.0	50.0	— 6.0	7.58	3.29	— 4.29
74.4	63.0	—11.4	9.04	5.30	— 3.74
74.4	65.4	— 9.0	16.63	6.21	—10.42
72.0	61.6	—10.4	8.40	3.38	— 5.02
78.0	63.0	—15.0	3.93	5.48	+ 1.55
59.6	51.0	— 8.6	19.46	11.69	— 7.77
52.0	45.6	— 6.4	9.04	5.62	— 3.42
47.8	39.4	— 8.4	8.45	4.25	— 4.20
51.2	47.8	— 3.4	6.76	3.79	— 2.97
78.8	63.0	—15.8	8.59	4.93	— 3.66
59.6	45.8	—13.8	7.93	5.39	— 2.54
59.6	50.6	— 9.0	9.04	4.48	— 4.56
71.2	72.0	+ 0.8	7.64	8.40	+ 0.76
					cow* excited
60.6	63.0	+ 2.4	4.69	5.33	+ 0.64
		Avg of lactating cows			dry cow
65.3	56.5	— 8.8	9.08	5.44	— 3.64

*Not included in average.

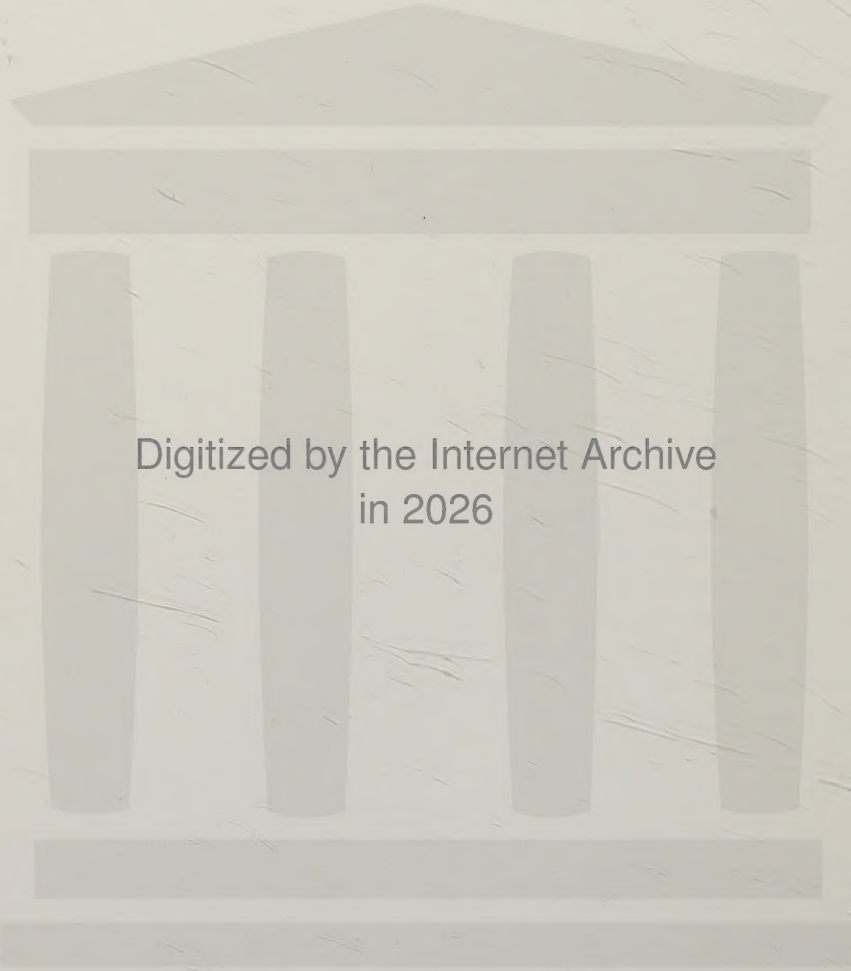
age lactose in the milk indicates a ratio of 550 volumes of blood for each unit volume of milk produced. It is, therefore, apparent that the glucose is not removed by the gland in sufficient quantities to account for all of the lactose in the milk. The volume flow of blood per unit volume of milk calculated from the combined average glucose and lactic acid differences as given in Table III and the average lactose percent in milk is about 390 which is in very good agreement with the values calculated from calcium and fat as cited above. Thus the combined glucose and lactic acid loss to the mammary gland appears to be sufficient to account for all of the milk sugar.

This confirms in part the observation by Graham² that the blood glucose is not the sole precursor of lactose and that lactic acid is also one of the precursors of lactose. Graham conducted a carbohydrate balance experiment on the mammary gland of a goat. While very good agreement was obtained in the two experiments reported, many more data are essential for conclusive proof.

Conclusions. 1. There was no apparent relation between arterio-venous blood sugar differences and the following: period of time following milking, arterial blood sugar level and level of milk production. 2. Both glucose and lactic acid are absorbed by the lactat-

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ing mammary gland in considerable quantities. While the glucose arterio-venous loss is not sufficient to account for all of the milk sugar, the combined loss of the two substances to the lactating mammary gland can account for all of the milk lactose.



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Amino Acids and other Non-Protein Nitrogen Blood Substances in Relation to Milk Secretion.

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Since Cary¹ demonstrated that the amino-acid content of blood obtained from the mammary vein of lactating cows was significantly lower than that obtained from the jugular vein, it has been assumed that the amino-acids of the blood are the precursors of the milk proteins. Recently Graham,² and Shaw and Petersen³ have presented evidence that the blood amino-acids are not the sole precursors of milk proteins. Graham, *et al.*,⁴ have also demonstrated that the blood proteins may be involved in the protein metabolism of the mammary gland.

Data are presented in this paper which show that the amino-acids of the blood cannot account for more than 30 to 40% of the milk proteins.

Arterial and mammary venous bloods were obtained and handled as reported by Shaw, Boyd and Petersen.⁵

Amino-acids were determined in tungstomolybdic acid filtrates by

* The data in this paper are from a thesis presented by J. C. Shaw in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, Minnesota Agricultural Experiment Station.

¹ Cary, C. A., *J. Biol. Chem.*, 1920, **43**, 477.

² Graham, W. R., Jr., *J. Biol. Chem.*, 1937, **122**, 1.

³ Shaw, J. C., and Petersen, W. E., *Proc. Am. Physiol. Soc.*, March 30, 31, April 1, 2, 1938, 50th Annual Meeting, Baltimore, Maryland, p. 183.

⁴ Graham, W. R., Jr., Peterson, V. R., Houchin, O. B., and Turner, C. W., *J. Biol. Chem.*, 1938, **122**, 275.

⁵ Shaw, J. C., Boyd, W. L., and Petersen, W. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 579.

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the colorimetric method of Folin.⁶ The apparent creatine and creatinine in blood were determined on the same filtrate by the method of Folin and Wu.⁷ Uric acid was determined on blood plasma by Benedict's direct colorimetric method.⁸ The Clark-Collip⁹ modification of the Kramer-Tisdall method was employed for the determination of calcium. Urea was determined on blood plasma samples by the urease method of Van Slyke and Cullen.^{10, 11}

Cary¹ made no attempt to determine the quantity of amino-acids which would have to be removed by the mammary gland to account for all of the proteins of the milk. In order to obtain a more complete picture of the nitrogen metabolism of the gland several non-protein fractions were determined on simultaneously drawn arterial and mammary venous bloods. Of these, herein are reported amino-acids; uric acid, urea, creatine and creatinine. Data are presented in Table I comparing the amino-acid and calcium arterio-venous difference in the passage of the blood through the mammary gland.

Amino-acids were removed from the blood by the gland in fairly constant amounts with an extreme variation of from 0.15 mg % to 0.97 mg %. The average arterio-venous difference was 0.46 mg %. On the basis of the protein in the milk this indicates that approximately 1200 volumes of blood per unit volume of milk would be necessary to supply enough amino-acids to account for the milk proteins if the amino-acids were the sole precursors of milk proteins. The average calcium loss of 0.32 mg % indicates that the volume flow of blood per unit volume of milk is approximately 390.

This is in good agreement with similar ratios calculated from fat and carbohydrate A-V differences and the quantity of fat and carbohydrate in the milk, Shaw and Petersen.³ It is evident then that the amino-acid absorption by the mammary gland is much too small to account for all of the milk proteins. If all of the amino-acids absorbed by the mammary gland from the blood were converted into milk proteins there would still be about 65% of the milk proteins to be accounted for by some other nitrogen substances in the blood. Graham² conducted a carbohydrate balance experiment on the mammary gland and reported 2 observations on amino-acids in which the amino-acid loss when compared to the glucose and lactic acid loss appeared to be too small to account for the milk proteins. In a

⁶ Folin, O., *J. Biol. Chem.*, 1922, **51**, 377.

⁷ Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

⁸ Benedict, S. R., *J. Biol. Chem.*, 1922, **51**, 187.

⁹ Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

¹⁰ Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, **19**, 211.

¹¹ Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1916, **24**, 117.

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TABLE I.
Amino Nitrogen in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods of Cows as Related to Calcium A-V Differences.

Amino nitrogen level		Plasma calcium		Remarks
Arterial, mg %	Venous, mg %	A-V difference, mg %	A-V difference, mg %	
3.92	3.25	—0.66	—0.31	2 hr. after milking
4.38	3.86	—0.52	—0.24	
3.72	3.26	—0.46	—0.54	
3.85	3.36	—0.49	—0.35	
3.08	2.93	—0.15	—0.19	3 " " "
4.23	3.72	—0.51	—0.30	
3.33	3.04	—0.29	—0.20	
3.76	3.23	—0.53	—0.32	4 " " "
3.30	3.05	—0.25	—0.46	
3.90	3.25	—0.65	—	
4.49	4.08	—0.31	—0.51	9 " " "
4.40	4.09	—0.31	—	
4.13	3.97	—0.16	—0.14	
4.28	3.31	—0.97	—0.29	
3.53	3.51	—0.02	—0.07	dry cow
Avg of lactating cows 3.90	3.44	—0.46	—0.32	

later paper Graham, *et al.*,⁴ presented experimental data indicating that the blood proteins were involved in the protein metabolism of the mammary gland.

The fact that the amino-acids cannot account for the major portion of the milk proteins lends support to the hypothesis that the blood proteins are involved in the elaboration of milk proteins.

TABLE II.
Uric Acid in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods.

Cow No.	Uric acid level		Arterial Venous Difference, mg %
	Arterial, mg %	Venous, mg %	
241	1.50	1.32	—0.18
445	1.72	1.30	—0.42
235	1.39	1.34	—0.05
432	1.50	1.65	+0.15
447	1.64	1.55	—0.09
419	1.79	1.72	—0.07
433	1.80	1.73	—0.07
23	1.43	1.36	—0.07
25	1.41	1.41	0
A-7	1.76	1.82	+0.06
586	1.75	1.79	+0.04
Avg	1.60	1.55	—0.05

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The data presented in Table II show that only very small amounts of blood uric acid are removed by the lactating mammary gland. In 11 analyses there was an average uric acid loss of -0.05 mg %.

Data are given in Table III on the arterio-venous creatinine and creatine differences. While there was a loss of creatinine and creatine in the majority of cases, the amounts absorbed by the mammary gland were extremely small. The average loss of each was 0.06 mg %.

TABLE III.
"Apparent" Creatine and Creatinine in Simultaneously Drawn Samples of
Arterial and Mammary Venous Bloods.

Cow No.	Creatinine level		Arterial Venous Difference, mg %	Creatine level		Arterial Venous Difference, mg %
	Arterial, mg %	Venous, mg %		Arterial, mg %	Venous, mg %	
443	1.29	1.20	— .09	2.23	2.16	— .07
445	1.56	1.35	— .21	1.63	1.75	+ .12
235	1.31	1.28	— .03	1.73	1.73	0
432	1.49	1.45	— .04	2.12	2.06	— .06
447	1.37	1.35	— .02	2.23	2.04	— .19
577	1.07	1.07	0	2.07	1.88	— .19
434	1.25	1.21	— .04	2.03	2.04	+ .01
241	1.20	1.12	— .08	—	—	—
419	1.27	1.25	— .02	—	—	—
440	1.22	1.16	— .06	—	—	—
Avg	1.30	1.24	— .06	2.01	1.95	— .06

Conclusions. 1. The amino-acids absorbed from the blood by the mammary cannot account for more than 35% of the milk proteins. 2. While there is a slight loss of blood uric acid, creatine and creatinine to the lactating mammary gland, it is evident that these substances are not important in the nitrogen metabolism of the lactating gland.

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PUBLICATIONS—

In-vitro Synthesis of Lactose (with W. E. Petersen). *Science*, 86:398. 1937.

The Ratio of Arterio-venous Differences of Certain Substances to Quantities Secreted by the Mammary Gland (with W. E. Petersen). *Proc. Am. Physiol. Soc.*, 50th Annual Meeting, March 30-April 2, 1938, p. 183.

Blood Glucose and Lactic Acid in Relation to Milk Secretion (with W. L. Boyd and W. E. Petersen). *Proc. Soc. Exp. Biol. and Med.*, 38:579. 1938.

Arginase in the Mammary Gland (with W. E. Petersen). *Proc. Soc. Exp. Biol. and Med.*, 38:631-632. 1938.

Amino Acids and Other Non-protein Nitrogen Blood Substances in Relation to Milk Secretion (with W. E. Petersen). *Proc. Soc. Exp. Biol. and Med.*, 38:632-635. 1938.

Fat Metabolism of the Mammary Gland (with W. E. Petersen). *Jour. Dairy Science*, 21:122. 1938.

Relation of Lactic Acid and Glucose of the Blood and Glycogen in the Mammary Gland (with W. E. Petersen). *Jour. Dairy Science*, 21:168. 1938.

